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Sent: Tue 4/19/2016 12:00:30 AM
Importance: Normal
Subject: BUGS
Received: Tue 4/19/2016 12:00:30 AM
BUGS LAB.docx

BUGS LAB

Investigation of PCR testing Procedures

An investigation of a failed Proficiency Testing (PT) for HIV lead to the conclusion that the procedure for testing was not being followed (the specimens were not vortexed) and that the CLA were overly involved in testing with questionable supervision. To investigate, I reviewed data put together for the Analyte Health investigation; several dozen positive Chlamydia, Gonorrhea and HIV cases that occurred over the last year or so together with maintenance records and package inserts. I reviewed proficiency testing for HIV as well as HCV, HBV and CT/NG and I spoke with Fran and Calvin Lung.

A fundamental issued with the BUGS lab is that they are physically split between upstairs and downstairs. Testing is all done downstairs with office work done upstairs. Specimens arrive from accessioning and are processed for testing by the CLA. For urine cultures, the CLA does the initial set-up including plating. For PCR testing, the CLA prepares the specimens and reagents for the CLS to do the set-up, which involves pipetting. Working together with the CLS, the samples are loaded onto the instrument for the first phase of testing. After the first phase, the instrument has reduced the specimens to a micro well plate. This is physically transferred to the next phase (a different instrument in a different room) by the CLA. After this phase of testing, the results of the run are downloaded into the computer and the CLS can evaluate the run and sign it out. Everyone agrees that this last step is only performed by the CLS.

Maintenance is performed by the CLA with supervision by the CLS. In reviewing the maintenance schedule, much of the required "maintenance" is little more than clean-up and daily disinfection. Some, however is technical and clearly more challenging. There is a significant question of which, if any, of these tasks can be performed by the CLA without supervision. There is also a significant question about how much supervision has been provided in this situation over the last couple of years.

A review of the patient reports with the associated run data appears in order. There are a couple of problems that have already been addressed. First the "lack of positive controls". The run for CT/NG have a negative control, a cut-off calibrator run in duplicate and a positive internal control. There is no external positive control. This was cited in our recent inspection and has been addressed. The second is the fact that the report lists an "operator" as the CLA. The CLS appends their initials to the plate name, and then not all the time. This is certainly confusing and a poor way of documenting their involvement.

A review of PT for 2015/16 from all of the related PCR assays (HIV, HBV, HCV and CT/NG) reveals the previously described failure for HIV viral load HIV-A 2016. This is a positive bias failure, with 3/5 results

narrowly exceeding the upper limit of the test. A fourth test is at +1.9 SDI and the fifth, which had an intended 0 value was correctly reported as such. (PT CA report filed). The only other PT failure was an isolated HCV viral load failure on HVL-C 2015. One of five tests was unacceptable, but again here we see that this is a bias failure with a negative bias this time: SDI ranging from 0 to -3.8 on the test that was out. Again we see the SDI of 0 was from a specimen with an intended value of 0, and this was correctly reported. The other tests all had significant negative bias. The finding of one test being out in a year is not unexpected and in and of itself is not unusual. I am more concerned with the implication to HIV in that there is a pattern of bias failures, and that the pattern of one positive bias failure and one negative bias failure is compatible with a mixing problem.

All of these problems add up to cast doubt on exactly how much supervision the CLS is providing during specimen set-up and instrument maintenance. Calvin Lung, in a direct interview, tells me that there is indeed supervision during that phase of testing. He does admit that they are now making procedural changes to ensure that supervision occurs, saying that in the past they were not very strict about this issue. He doe reiterate that supervision was always present at some level. As to the issue of vortexing or mixing samples by inversion, Calvin tells me that a representative from Abbott had verbally instructed them to mix by inversion to avoid bubbles. In reviewing the procedures, all state to mix by vortexing, although at least one cautions against bubbles and requires that any foam generated by vortexing be pipetted off. Subsequent written clarification from Abbott states that vortexing is necessary and inversion is not an adequate substitute.

Action items:

1) Retrain personnel on HIV assay, re: vortexing.
2) Perform patient assessment for HIV viral load assay.
3) Retrain personnel for HBV, HCV and CT/NG assay, re: vortexing.
4) Re-write job description for CLA in BUGS Lab making it clear that specimen preparation and instrument maintenance can only be performed with direct supervision.
5) Re-write maintenance procedures making it clear that general clean up and routine disinfection can be performed by CLA without direct CLS supervision.